Coxsackie Virus in Southern California

Isolation of a Strain from Stools of a Patient

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• *Thirty-three stool specimens from 29 patients were examined for Coxsackie virus by the inoculation of suckling mice. Such a virus, designated "California I," was obtained from two stool specimens collected on successive days from a patient with so-called nonparalytic poliomyelitis.*

Neutralizing antibodies for the California I strain of Coxsackie virus could not be demonstrated in serum obtained from the patient early in the illness, but were present in convalescent serum.

Serum from the patient's daughter, who previously had had a similar illness, neutralized the strain of virus isolated from the father.

In pathologic examination of the skeletal muscles of mice infected with the California I virus, lesions typical of those produced by Coxsackie virus, group A, were noted.

California I strain of the virus was not neutralized by immune serum prepared from several other strains of Coxsackie virus.

WHILE STUDYING several small epidemics of poliomyelitis in upstate New York in 1947, Dalldorf and Sickles[3] isolated a virus by the intracerebral injection of suckling mice and hamsters with supernates from fecal specimens obtained from two children living in Coxsackie, N. Y. The virus, now designated "Coxsackie virus," produced paralysis and death in the mice and hamsters. Upon pathologic examination widespread degeneration of the skeletal muscles was observed. In the same year, in Wilmington, Del., an outbreak of atypical poliomyelitis occurred in which multiple infections were observed in families and the paralysis was transient in many cases. Furthermore, several investigators failed to isolate the classic virus of poliomyelitis. Dalldorf[4] examined 21 specimens of feces from patients in the area and in five instances again recovered a virus that produced paralysis and death in suckling mice.

During the years 1947, 1948, and 1949, Dalldorf and Gifford[5] examined 517 stool specimens from patients in large and small epidemics of poliomyelitis and from a few sporadic cases. Coxsackie virus was obtained from 30 (5.8 per cent) of the specimens. Dalldorf[6] observed that most of the strains of virus produced extensive generalized degeneration of striated muscles in mice, and a few strains caused, in addition to the muscle degeneration, lesions in the central nervous system. Those that caused only muscle degeneration were classified as group A viruses, and those that caused lesions in the central nervous system were classified as group B. In 22 of the previously mentioned 30 cases the viruses isolated were observed to be group A, and some paralysis had developed in 14 of the patients. In the remaining eight cases the viruses were group B, and none of the patients was paralyzed.

Melnick, Shaw and Curnen, [13, 16] isolated a similar virus from patients. They detected Coxsackie viruses in sewage and flies and described cases of the disease among laboratory personnel working with the virus. Subsequently, the virus was isolated in other areas of the United States,[2, 8, 9, 11, 15] also in Canada,[1, 9] Denmark[12] and England.[7] Slater and Syverton[17] in 1950 isolated Coxsackie virus from a patient with nonparalytic poliomyelitis in Minnesota and maintained the virus in tissue culture for 24 successive passages.

Coxsackie virus produces a variety of symptoms; namely, fever, headache, backache, muscle pain and spasm, stiff neck, abdominal pain, nausea, vomiting, pleural pain, sore throat, malaise and anorexia. The most consistent symptoms are fever and headache, yet they vary in different epidemics. Paralysis occurs in a small percentage of cases. The clinical laboratory findings are limited chiefly to pleocytosis of the cerebrospinal fluid. Two rather distinct clinical syndromes, other than that resembling poliomyelitis, have been associated with the virus. Findlay and Howard[7] in England recovered the virus from patients with so-called epidemic pleurodynia, which has been known as Bornholm disease. Hueb-

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ner and co-workers[10] reported isolation of group A Coxsackie virus from 32 patients with herpangina in Maryland.

Coxsackie virus has been detected in several areas of the United States, but to date it has not been reported in the southwestern states with the exception of Texas. The present study was undertaken to determine if the virus could be detected in California. Poliomyelitis is endemic in the Los Angeles area throughout the year and the so-called nonparalytic form of the disease is prevalent.

MATERIALS AND METHODS

Stool specimens and paired blood samples were collected during the months of May, June, July and August 1951 from 29 patients at the Long Beach Veterans Administration Hospital with tentative diagnoses including septic meningitis, nonparalytic poliomyelitis, pneumonitis, and ulcerative colitis. The sera for neutralization tests and the stool specimens were stored at —70° C. The inoculum was prepared from the stool specimen, after thawing, by making a 20 per cent suspension in distilled water. The suspension was ground in a Ten Broeck grinder and centrifuged in a Spinco for one-half hour at 13,000 rpm. The supernate was exposed for one-half hour at room temperature to 1,000 units of penicillin and 10.0 mg. of streptomycin per milliliter. Three-hundredths of a milliliter of the inoculum was injected either intramuscularly or intraperitoneally into mice from one to two days old. The mice were observed at frequent intervals and whenever paralysis appeared they were removed and immediately frozen at —70° C. Inoculated mice that had nervous symptoms or that appeared unthrifty were sacrificed and washed with ether. Subsequently, the feet, tail, head, skin and viscera were removed and the remaining skeletal tissue finely minced for repeated animal passage. A 20 per cent suspension of tissue in distilled water was prepared in a Ten Broeck grinder, and centrifuged at 1,000 rpm. in a cold room for five minutes. Groups of suckling mice were injected either intramuscularly or intraperitoneally with 0.03 ml. of the supernate. In a few instances, blind passages were repeated several times.

Identification of the virus was attempted by neutralization tests using immune sera prepared from known strains of Coxsackie virus. The immune serum was produced by inoculating three-week-old mice intramuscularly or intraperitoneally with 0.1 ml. of a 10 per cent suspension of infected muscle-bone. Two inoculations were made each week for three weeks. After a rest period of one week, a single inoculation was made. The mice were bled two weeks later. The neutralization tests described by Melnick and Ledinko[14] were carried out.

The suckling mice were obtained from two separate colonies of white mice. They were pooled from several litters and redistributed to the mothers to avoid the use of one litter for a single inoculum. The inoculated mice were isolated in special quarters distant from the normal mouse colony and from the laboratory in which mice infected with known strains of virus were housed. At various times, litters from the two normal mouse colonies were tested for Coxsackie virus and always found to be negative.

RESULTS

The inoculum from only two of 33 stool specimens produced paralysis in suckling mice. The two specimens were obtained from the same patient on the sixth and seventh days of illness. All of a group of ten mice injected with inoculum from the first specimen, and all of a group of six given inoculum from the second specimen had paralysis in five days. Inoculum from a third specimen obtained from the patient 20 days after the onset of illness did not produce paralysis in a litter of six one-day-old mice.

The patient from whom the specimens were obtained was a 32-year-old white male cement mill operator. He was well until the day before admission to the hospital. The illness began with fever, chills, headache and backache, with stiff neck, nausea and vomiting on the second day. The acute illness continued for five days with fever as high as 103.6° F. Pain in the chest was present during the first week of illness. Nuchal rigidity persisted for 14 days. During the first two weeks of illness there were from 75 to 750 lymphocytes per cu. mm. of cerebrospinal fluid. The patient was discharged 19 days after admission to the hospital and remained well. As the patient had said that his eight-year-old daughter had likewise been ill with similar symptoms—sore throat, fever, headache, stiff neck—three weeks before he himself had become ill, serum was obtained from her and it neutralized the virus isolated from the father.

The virus isolated from the two stool specimens from the patient on successive days was not neutralized by immune sera prepared from Dalldorf, type I, II, III, Highpoint, Texas, and Connecticut 5 strains, but was neutralized by a homologous antiserum. Serum obtained from the patient early in the illness was ineffective but serum collected 20 days after the onset of the disease neutralized the virus. The virus has been designated as "California I."

In a pathologic examination of the skeletal muscles of mice infected with the California I strain, extensive degeneration was observed. The lesions were typical of those produced by group A Coxsackie virus. No lesions were noted in the tissues of the central nervous system.

DISCUSSION

Although a virus, identified as of Coxsackie type, was isolated from only two of 33 stool specimens obtained from 29 patients, it should be pointed out that the patients were adults ranging in age from 28 to 60 years, an age group older than that in which the virus is most prevalent. In the series reported upon by Dalldorf and Gifford,[5] 73 per cent of the patients were under ten years of age. Although no reports have appeared in the literature that Coxsackie viruses have been isolated in California, they would be expected to be as prevalent here as elsewhere. Virologists have not made a diligent search. The constant endemicity of poliomyelitis in Southern California is well known. Inasmuch as one form of illness resulting from infection with Coxsackie virus is similar to poliomyelitis, it may, in some cases, be erroneously diagnosed as poliomyelitis. The differentiation of the two diseases is dependent upon the intracerebral inoculation of monkeys to determine whether the virus of poliomyelitis is or is not present.

In the case of the patient from whom California I virus was obtained, it is probable that the patient's daughter had been infected by the same virus three weeks earlier than he, inasmuch as she had similar symptoms and serum obtained from her neutralized the virus obtained from him.

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